

I claim:

1. A method for quantifying expression of one or more target genes in living cells, comprising:

(a) providing cells that possess:

5 (1) at least a first fluorescently labeled RNA binding polypeptide, wherein the first fluorescently labeled RNA binding polypeptide comprises a first RNA binding domain; and

(2) at least a first target gene of interest, where the target gene has been modified to comprise one or more nucleic acid sequences encoding a first binding site for
10 the first RNA-binding domain, wherein upon expression of the first target gene into a first target RNA, the first binding site is specifically bound by the first fluorescently labeled RNA-binding polypeptide;

(b) scanning the cells to obtain fluorescent signals from the first fluorescently labeled RNA binding polypeptide;

15 (c) determining fluorescent emission intensities from the first fluorescently labeled RNA binding polypeptide at two different wavelengths;

(d) calculating a ratio of the fluorescent emission intensities from the first fluorescently labeled RNA binding polypeptide at the two different wavelengths; and

(e) calculating a quantity of the first target RNA in the cells from the ratio.
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2. The method of claim 1 wherein the first fluorescently labeled RNA binding polypeptide further comprises a nuclear export signal.

3. The method of claim 2 wherein the nuclear export signal comprises an amino acid
25 sequence of the general formula:

XXXLXXLXL, where X is any amino acid (SEQ ID NO:30).

4. The method of claim 3 wherein the nuclear export signal comprises an amino acid sequence selected from the group consisting of:

30 ALQKKLEELDE (SEQ ID NO:19);

DLQKKLEELDE (SEQ ID NO:20);

LPPLERLTL (SEQ ID NO:23);
LQQQLGQLTL (SEQ ID NO:25);
LDKLSVLTLS (SEQ ID NO:27); and
LWQFLLQLLLD (SEQ ID NO:28).

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5. The method of claim 2 wherein the nuclear export signal comprises an amino acid sequence selected from the group consisting of :

DKERWEDVKEEMTSALATMRVDYE (SEQ ID NO:21);
WDRTFSLFQQLQSSFVVE (SEQ ID NO:22);
10 LALKLAGLDI (SEQ ID NO:24);
LESNLRELQI (SEQ ID NO:26); and
LCQAFSKVILA (SEQ ID NO:29).

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6. The method of claim 2 wherein the first fluorescently labeled RNA binding polypeptide comprises a fluorophores pair selected from the group consisting of:

- a) a donor/acceptor pair for fluorescence resonance energy transfer;
- b) an excimer forming-pair; and
- c) an exciplex-forming pair.

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7 The method of claim 6 wherein the fluorophore pair is a donor/acceptor pair for fluorescence resonance energy transfer.

8 The method of claim 7 wherein the donor/acceptor pair is selected from the group consisting of:

25 fluorescein/rhodamine;
fluorescein/eosin;
fluorescein/erythrosine;
fluorescein/QSY-7;
fluorescein/ALEXA FLUOR® 54;
30 fluorescein/BODIPY®-TMR Cy3;
fluorescein/ALEXA FLUOR® 532;

ALEXA FLUOR® 488/rhodamine;

ALEXA FLUOR® 488/eosin;

ALEXA FLUOR® 488/erythrosine;

ALEXA FLUOR® 488/QSY-7;

5 ALEXA FLUOR® 488/ALEXA FLUOR® 54;

ALEXA FLUOR® 488/ BODIPY®-TMR Cy3;

ALEXA FLUOR® 488/ALEXA FLUOR® 532;

ALEXA FLUOR® 532 /ALEXA FLUOR® 546;

ALEXA FLUOR® 532/rhodamine;

10 ALEXA FLUOR®350/ALEXA FLUOR® 430;

ALEXA FLUOR®430/ALEXA FLUOR® 532;

ALEXA FLUOR®430/eosin;

ALEXA FLUOR®430/rhodamine; and

ALEXA FLUOR®430/BODIPY®-TMR Cy3.

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9. The method of claim 6 wherein the fluorophore pair is a excimer-forming pair.

10. The method of claim 9 wherein the excimer-forming pair is selected from the group consisting of:

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a) a pyrene pair; and

b) a BODIPY-FL® pair.

11. The method of claim 6 wherein the fluorophore pair is a exciplex-forming pair.

25 12. The method of claim 11 wherein the exciplex-forming pair consists of anthracene and diethylaniline.

13. The method of claim 7 wherein the ratio of emission intensities comprise a ratio of emission intensity for the donor and an emission intensity for the acceptor.

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14. The method of claim 9 wherein the ratio of the emission intensities comprises a ratio of an emission intensity of the excimer pair and an emission intensity of a monomer of either of the non-covalently interacting fluorophores.
- 5 15. The method of claim 11 wherein the ratio of the emission intensities comprises a ratio of an emission intensity of the exciplex pair and an emission intensity of a monomer of either of the non-interacting fluorophores.

16. The method of claim 2 wherein the first fluorescently labeled RNA binding polypeptides are membrane permeant.
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17. The method of claim 16 wherein the RNA binding domain comprises an amino acid sequence selected from the group consisting of:

TRQARRNRRRWRERQR (SEQ ID NO:6);
{M/L)DAQTRRRERRAEKQAQWK (SEQ ID NO:8);
NAKTRRHERRRKLAIER (SEQ ID NO:10);
MPKTRRRPRRSQRKRP (SEQ ID NO:12); and
GRKKRRQRRRPPQ (SEQ ID NO:14).

18. The method of claim 1 wherein the first RNA binding domain comprises an amino acid selected from the group consisting of

- (i) MDAQTRRRERRAEKQAQWKAANKG (SEQ ID NO:31);
(ii) MDAQTRRRERRAEKQAQWKAANK (SEQ ID NO:32);
(iii) MDAQTRRRERRAEKQAQWK (SEQ ID NO:33);
(iv) MDAQTRRRERRAEKQAQWKA (SEQ ID NO:34);
(v) MDAQTRRRERRAEKQAQWCAA (SEQ ID NO: 35);
(vi) MDAQTRRRERRAEKQAQWKAAN (SEQ ID NO: 36);
(vii) LDAQTRRRERRAEKQAQWKAANKG (SEQ ID NO:37);
(viii) LDAQTRRRERRAEKQAQWKAANK (SEQ ID NO:38);
(ix) LDAQTRRRERRAEKQAQWK (SEQ ID NO:39);

- (x) LDAQTRRRERRAEKQAQWKA (SEQ ID NO:40);
- (xi) LDAQTRRRERRAEKQAQWKAA (SEQ ID NO:41); and
- (xii) LDAQTRRRERRAEKQAQWKAAN (SEQ ID NO:42).

5 19. The method of claim 18, wherein the target RNA comprises a nucleotide sequence of the general formula NNGC(C/G)CUG(G/A)(G/A)(G/A)AAGGGCRR, wherein N is G or is absent and R is C or is absent (SEQ ID NO:9).

10 20. The method of claim 1 wherein the first RNA binding domain comprises an amino acid sequence TRQARRNRRRRWRERQR (SEQ ID NO:6).

21. The method of claim 20 wherein the target RNA comprises a nucleotide sequence GGUCUGGGCGCAGCGCAAGCUGACGGUACA (SEQ ID NO:7).

15 22. The method of claim 1 wherein the first RNA binding domain comprises an amino acid sequence NAKTRRHERRRKLAIER (SEQ ID NO:10).

23. The method of claim 22 wherein the target RNA comprises a nucleotide sequence of GGUGCGCUGACAAAGCGCGCC (SEQ ID NO:11).

20 24. The method of claim 1 wherein the first RNA binding domain comprises an amino acid sequence MPKTRRRPRRSQRKRP (SEQ ID NO:12).

25 25. The method of claim 24 wherein the target RNA comprises a nucleotide sequence of GGGCGCCGGUACGCAAGUACGACGGUACGCUC (SEQ ID NO:13).

26. The method of claim 1 wherein the first RNA binding domain comprises an amino acid sequence of GRKKRRQRRRPPQ (SEQ ID NO:14).

27. The method of claim 26 wherein the target RNA comprises a nucleotide sequence selected from the group consisting of GGCCAGAUCUGAGCCU (**SEQ ID NO:15**) and GGGAGCUCUCUGGCC (**SEQ ID NO:16**)

5 28. The method of claim 2 wherein the first fluorescently labeled RNA binding polypeptide comprises an amino acid sequence selected from the group consisting of:

RQIKIWFQNRRMKWKK (**SEQ ID NO:1**);

GALFLGWLGAAGSTMGAWSQPKKKRKV (**SEQ ID NO:2**);

AAVALLPAVLLALLAP (**SEQ ID NO:3**);

10 GWTLNSAGYLLKINLKALAALAKKIL (**SEQ ID NO:4**);

KLALKLALKALKAALKLA (**SEQ ID NO:5**); and

amino acid sequences of between 4 and 30 amino acids comprising between 4 and 12 arginine residues.

15 29. The method of claim 2 further comprising contacting a subset of the cells with one or more test compounds, and comparing the quantity of the target RNA in cells contacted with the one or more test compounds with the quantity of the target RNA in cells not contacted with the one or more test compound.

20 30. The method of claim 2 wherein the scanning comprises obtaining a visual representation of the fluorescent signals from the first fluorescently labeled RNA binding polypeptide.

25 31. The method of claim 2 further comprising determining the localization of the first fluorescently labeled RNA binding polypeptide within individual cells.

30 32. The method of claim 2 wherein the calculating fluorescent emission intensities from the first fluorescently labeled RNA binding polypeptide at two different wavelengths is performed at multiple time points.

33. The method of claim 2 wherein the cells comprise two or more distinct populations of cells, and wherein the calculating fluorescent emission intensities from the first fluorescently labeled RNA binding polypeptide at two different wavelengths is compared between the distinct cell populations.

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34. A fluorescently labeled RNA binding polypeptide, comprising:

(a) a non-naturally occurring amino acid sequence comprising

(i) a nuclear export signal; and

(ii) an RNA binding domain, wherein the amino acid; and

10 (b) a fluorophore pair selected from the group consisting of

(i) a donor/acceptor pair for fluorescence resonance energy transfer;

(ii) an excimer forming fluorophore pair; and

(iii) an exciplex forming fluorophore pair.

15 35. The fluorescently labeled RNA binding polypeptide of claim 35 wherein the amino acid sequence comprising an RNA binding domain is membrane permeant.

36. The fluorescently labeled RNA binding polypeptide of claim 34 wherein the nuclear export signal comprises an amino acid sequence of the general formula

20 XXXLXXLXL, where X is any amino acid (SEQ ID NO:30).

37. The fluorescently labeled RNA binding polypeptide of claim 36 wherein the nuclear export signal comprises an amino acid sequence selected from the group consisting of:

25 ALQKKLEELELDE (SEQ ID NO:19);

DLQKKLEELELDE (SEQ ID NO:20);

LPPLERLTL (SEQ ID NO:23);

LQQQLGQLTL (SEQ ID NO:25);

LDKLSVLTLS (SEQ ID NO:27); and

30 LWQFLLQLLLD (SEQ ID NO:28).

38. The fluorescently labeled RNA binding polypeptide of claim 34 wherein the nuclear export signal comprises an amino acid sequence selected from the group consisting of :

5 DKERWEDVKEEMTSALATMRVDYE (SEQ ID NO:21);
WDRTFSLFQQLQSSFVVE (SEQ ID NO:22);
LALKLAGLDI (SEQ ID NO:24);
LESNLRELQI (SEQ ID NO:26); and
LCQAFSKVILA (SEQ ID NO:29).

10 39. The fluorescently labeled RNA binding polypeptide of claim 35 wherein the RNA binding domain comprises an amino acid sequence selected from the group consisting of

15 TRQARRNRRRRWRERQR (SEQ ID NO:6);
MDAQTRRRERRAEKQAQWKAAN (SEQ ID NO:8);
NAKTRRHERRRKLAIER (SEQ ID NO:10);
MPKTRRRPRRSQRKRP (SEQ ID NO:12); and
GRKKRRQRRRPPQ (SEQ ID NO:14).

20 40. The fluorescently labeled RNA binding polypeptide of claim 39 wherein the fluorophore pair is a donor/acceptor pair for fluorescence resonance energy transfer, and wherein the donor/acceptor pair is selected from the group consisting of:

25 fluorescein/rhodamine;
fluorescein/eosin;
fluorescein/erythrosine;
fluorescein/QSY-7;
fluorescein/ALEXA FLUOR® 54;
fluorescein/BODIPY®-TMR Cy3;
fluorescein/ALEXA FLUOR® 532;
ALEXA FLUOR® 488/rhodamine;
ALEXA FLUOR® 488/eosin;
30 ALEXA FLUOR® 488/erythrosine;
ALEXA FLUOR® 488/QSY-7;

ALEXA FLUOR® 488/ALEXA FLUOR® 54;
ALEXA FLUOR® 488/ BODIPY®-TMR Cy3;
ALEXA FLUOR® 488/ALEXA FLUOR® 532;
ALEXA FLUOR® 532 /ALEXA FLUOR® 546;
5 ALEXA FLUOR® 532/rhodamine;
ALEXA FLUOR®350/ALEXA FLUOR® 430;
ALEXA FLUOR®430/ALEXA FLUOR® 532;
ALEXA FLUOR®430/eosin;
ALEXA FLUOR®430/rhodamine; and
10 ALEXA FLUOR®430/BODIPY®-TMR Cy3.

41. The fluorescently labeled RNA binding polypeptide of claim 39 wherein the fluorophore pair is an excimer-forming pair.

15 42. The fluorescently labeled RNA binding polypeptide of claim 41 wherein the excimer forming pair is selected from the group consisting of:
a) a pyrene pair; and
b) a BODIPY-FL® pair.

20 43. The fluorescently labeled RNA binding polypeptide of claim 39 wherein the fluorophore pair is an exciplex-forming pair.

44. The fluorescently labeled RNA binding polypeptide of claim 43 wherein the exciplex-forming pair consists of anthracene and diethylaniline.

25 45. The fluorescently labeled RNA binding polypeptide of claim 34, wherein the amino acid sequence further comprises an amino acid sequence selected from the group consisting of:

30 RQIKIWFQNRRMKWKK (SEQ ID NO:1);
GALFLGWLGAAGSTMGAWSQPKKKRKV (SEQ ID NO:2);
AAVALLPAVLLALLAP (SEQ ID NO:3);

GWTLNSAGYLLKINLKALAALAKKIL (SEQ ID NO:4);

KLALKLALKALKAAKLKLA (SEQ ID NO:5); and

amino acid sequences of between 4 and 30 amino acids comprising between 4 and 12 arginine residues.

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46. A fluorescently labeled RNA binding polypeptide comprising:

(a) an RNA binding domain consisting of an amino acid sequence selected from the group consisting of:

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(i) MDAQTRRRERRAEKQAQWKAANKG (SEQ ID NO:31);

(ii) MDAQTRRRERRAEKQAQWKAANK (SEQ ID NO:32);

(iii) MDAQTRRRERRAEKQAQWK (SEQ ID NO:33);

(iv) MDAQTRRRERRAEKQAQWKA (SEQ ID NO:34);

(v) MDAQTRRRERRAEKQAQWKAA (SEQ ID NO: 35);

(vi) MDAQTRRRERRAEKQAQWKAAN (SEQ ID NO: 36);

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(vii) LDAQTRRRERRAEKQAQWKAANKG (SEQ ID NO:37);

(viii) LDAQTRRRERRAEKQAQWKAANK (SEQ ID NO:38);

(ix) LDAQTRRRERRAEKQAQWK (SEQ ID NO:39);

(x) LDAQTRRRERRAEKQAQWKA (SEQ ID NO:40);

(xi) LDAQTRRRERRAEKQAQWKAA (SEQ ID NO:41); and

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(xii) LDAQTRRRERRAEKQAQWKAAN (SEQ ID NO:42); and

(b) a donor/acceptor fluorophore pair selected from the group consisting of:

fluorescein/rhodamine;

fluorescein/eosin;

fluorescein/erythrosine;

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fluorescein/QSY-7;

fluorescein/ALEXA FLUOR® 54;

fluorescein/BODIPY®-TMR Cy3;

fluorescein/ALEXA FLUOR® 532;

ALEXA FLUOR® 488/rhodamine;

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ALEXA FLUOR® 488/eosin;

ALEXA FLUOR® 488/erythrosine;

ALEXA FLUOR® 488/QSY-7;
 ALEXA FLUOR® 488/ALEXA FLUOR® 54;
 ALEXA FLUOR® 488/ BODIPY®-TMR Cy3;
 ALEXA FLUOR® 488/ALEXA FLUOR® 532;
 ALEXA FLUOR® 532 /ALEXA FLUOR® 546;
 ALEXA FLUOR® 532/rhodamine;
 ALEXA FLUOR®350/ALEXA FLUOR® 430;
 ALEXA FLUOR®430/ALEXA FLUOR® 532;
 ALEXA FLUOR®430/eosin;
 ALEXA FLUOR®430/rhodamine; and
 ALEXA FLUOR®430/BODIPY®-TMR Cy3.

47. A fluorescently labeled RNA binding polypeptide comprising:

(a) an RNA binding domain consisting of an amino acid sequence selected from the group consisting of:

- (i) MDAQTRRRERRAEKQAQWKAANKG (SEQ ID NO:31);
- (ii) MDAQTRRRERRAEKQAQWKAANK (SEQ ID NO:32);
- (iii) MDAQTRRRERRAEKQAQWK (SEQ ID NO:33);
- (iv) MDAQTRRRERRAEKQAQWKA (SEQ ID NO:34);
- (v) MDAQTRRRERRAEKQAQWKAA (SEQ ID NO: 35);
- (vi) MDAQTRRRERRAEKQAQWKAAN (SEQ ID NO: 36);
- (vii) LDAQTRRRERRAEKQAQWKAANKG (SEQ ID NO:37);
- (viii) LDAQTRRRERRAEKQAQWKAANK (SEQ ID NO:38);
- (ix) LDAQTRRRERRAEKQAQWK (SEQ ID NO:39);
- (x) LDAQTRRRERRAEKQAQWKA (SEQ ID NO:40);
- (xi) LDAQTRRRERRAEKQAQWKAA (SEQ ID NO:41); and
- (xii) LDAQTRRRERRAEKQAQWKAAN (SEQ ID NO:42); and

(b) a nuclear export signal consisting of an amino acid selected from the group consisting of:

- (i) XXXLXXLXL, where X is any amino acid (SEQ ID NO:30);
- (ii) ALQKKLEELDE (SEQ ID NO:19);
- (iii) DLQKKLEELDE (SEQ ID NO:20);

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- (iv) LPPLERLTL (SEQ ID NO:23);
 - (v) LQQQLGQLTL (SEQ ID NO:25);
 - (vi) LDKLSVLTLS (SEQ ID NO:27);
 - (vii) LWQFLQLLLD (SEQ ID NO:28);
 - (viii) DKERWEDVKEEMTSALATMRVDYE (SEQ ID NO:21);
 - (ix) WDRTFSLFQQLQSSFVVE (SEQ ID NO:22);
 - (x) LALKLAGLDI (SEQ ID NO:24);
 - (xi) LESNLRELQI (SEQ ID NO:26); and
 - (xii) LCQAFSKVILA (SEQ ID NO:29); and
- 10 (c) a donor/acceptor fluorophore pair selected from the group consisting of:
- fluorescein/rhodamine;
 - fluorescein/eosin;
 - fluorescein/erythrosine;
 - fluorescein/QSY-7;
 - fluorescein/ALEXA FLUOR® 54;
 - fluorescein/BODIPY®-TMR Cy3;
 - fluorescein/ALEXA FLUOR® 532;
 - ALEXA FLUOR® 488/rhodamine;
 - ALEXA FLUOR® 488/eosin;
 - ALEXA FLUOR® 488/erythrosine;
 - ALEXA FLUOR® 488/QSY-7;
 - ALEXA FLUOR® 488/ALEXA FLUOR® 54;
 - ALEXA FLUOR® 488/ BODIPY®-TMR Cy3;
 - ALEXA FLUOR® 488/ALEXA FLUOR® 532;
 - ALEXA FLUOR® 532 /ALEXA FLUOR® 546;
 - ALEXA FLUOR® 532/rhodamine;
 - ALEXA FLUOR®350/ALEXA FLUOR® 430;
 - ALEXA FLUOR®430/ALEXA FLUOR® 532;
 - ALEXA FLUOR®430/eosin;
 - ALEXA FLUOR®430/rhodamine; and
 - ALEXA FLUOR®430/BODIPY®-TMR Cy3.
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